Hypotensive Action of *Solanum melongena* on Normotensive Rats

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The cardiovascular action of *Solanum melongena* extract (SME) was investigated using *in vivo* and *in vitro* preparations. SME produced dose-dependent hypotensive responses in normotensive albino rats. The duration of response was also dose dependent. In pharmacological antagonist studies, the hypotensive action of SME was proved not to be mediated through the autonomic ganglion, the α-adrenoceptor or the histaminergic receptor. SME worked via the β-adrenoceptor and cholinergic (muscarinic) receptor inducing the hypotensive response. A dose-related attenuation of hypotension with increasing dosages of the β-adrenoceptor blocker, propranolol, and the cholinergic receptor blocker, atropine was observed. *In vitro* studies indicated that the vasorelaxing and negative inotropic effect of SME might be implicated in the hypotensive response. The activities of the β-adrenergic and acetylcholine receptors mediated by SME in turn promoted vasodilation of the resistant vessels and a reduction in cardiac activity respectively. It is also possible that the hypotensive effect of SME could be accounted for by its influence on the activity of the renin–angiotensin system, since a significant difference of the hypotensive response of SME was obtained with captopril. Furthermore, SME induced diuresis in water-loaded rats which might also account for the hypotensive effect observed. SME could be a very potent hypotensive agent.

Keywords: Solanum melongena; hypotensive action.

INTRODUCTION

The plants genus *Solanum* contain various steroidal glycoalkaloids which structrually resemble the veratrum alkaloid (Craig and Jacobs, 1943), the latter producing effects of hypotension, bradycardia, depression of artificially stimulated heart (antiaccelerator effect) and respiratory arrest in intact animals (Krayer and Acheson, 1946). *Solanum melongena*, known in the United States as eggplant and originating in India, has been recognized for its culinary and pharmacological properties since ancient times (Pistoia and Dejey, 1977; Wu Nan Research Institute of Chinese Medicine, 1977).

Reportedly, the fruit possesses diuretic and cholagogic properties (Pistoia and Dejey, 1977; Wu Nan Research Institute of Chinese Medicine, 1977; Kan, 1977). In this study, a hypotensive effect of the crude extract from fresh berries of *Solanum melongena* was reported and its putative mechanism of action was investigated by using *in vivo* and *in vitro* preparations.

MATERIALS AND METHODS

Preparation of *Solanum melongena* extract (SME)

Solanum melongena (200 g) was boiled in 2 L distilled water for about 4 h. Filtration using Whatman No. 1 filter paper under suction yielded 600 mL extract. This aqueous extract was concentrated to roughly 300 mL by a rotary evaporator at 50 °C. It was then dialysed (dialyser tube: Spectraper: 21 mm; 3787-032) at room temperature. The extract was further concentrated by rotovaporator to 300 mL and then lyophilized to yield 1.5 g SME in powder form for storage. The ion contents of 0.01 g/mL extract before and after dialysis were measured by the atomic absorption spectrometer: 553 μg/mL potassium ion and 3.91 μg/mL calcium ion in the non-dialysed extract and 104 μg/mL potassium ion and 0.25 μg/mL calcium ion in the dialysed extract were obtained.

In vivo preparation

Female Sprague-Dawley rats (200–250 g) were anaesthetized with 50 mg/kg sodium pentobarbitol by intraperitoneal injection. When the rat was unconscious, an incision on the skin from the lower jaw to the upper part of thorax was made. Cannulae were placed in the trachea to facilitate spontaneous respiration, in the left carotid artery for blood pressure recording and in the external jugular vein for SME administration. Mean arterial blood pressure (MAP) was recorded using a Narco P-1000B transducer on a MK-III Narco Physiograph (Narco Instrument, Texas) (Larochelle and Hamet, 1983).

Pharmacological antagonist studies

The autonomic ganglion transmission, α-, β-adrenergic, muscarinic cholinergic and histaminergic activities on the change of MAP with SME were examined by using their specific antagonists and agonists

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(Ho *et al.*, 1989). Their antagonists: hexamethonium (3 mg/kg, Sigma), phentolamine (2 mg/kg, Ciba), propranolol (2 mg/kg, Sigma), atropine (2 mg/kg, Sigma), pyrilamine (15 mg/kg, Sigma) as an H-1 receptor blocker and cimetidine (15 mg/kg, Sigma) as an H-2 receptor blocker were used. Methoxamine (100 μg/kg, Burroughs–Wellcome), isoproterenol (1.2 μg/kg, Sigma), acetylcholine (2 μg/kg, Merck) and histamine (2 μg/kg, Sigma) acted as α-, β-adrenoceptor, cholinergic and histaminergic receptor agonists, respectively. The protocol was as follows: the MAP of the rat preparation was first assessed by injection of an agonist in an effective dose. The appropriate antagonist was then introduced. When the MAP was stable, injection of the agonist was repeated to ensure the blockade effect of the antagonist. When the effect was attained, a test dose of SME (6.5 mg/kg) was given.

The possible interaction between the hypotensive action of SME and the renin–angiotensin system was studied using the converting enzyme inhibitor, captopril. Captopril (10 μg/min/kg) was infused into the rat through the femoral vein at a rate of 0.07 mL/min. Angiotensin I (40 ng/kg, Sigma), SME and bradykinin (10 μg/kg, Sigma) were tested separately before and after 30 min of captopril infusion. The decrease in MAP and also the duration for 75% recovery of the response were recorded (Sham, 1983).

Diuretic studies

The study of diuretic effect of SME was done by means of water-loading the female rats and infusion of SME with doses of 0.03, 0.1, 1, 3 mg/min in rats. Atrial natriuretic peptide (ANF, 500 ng/min, Bachem) was used as the reference drug. The rats were anaesthetized with sodium pentobarbital (i.p.). Infusion of 10 mM NaCl in 5% glucose through a tail vein at a rate of 0.2 mL per min was applied for 2 h. About 30 min after the emergence of the first drop of urine, a urine sample was collected as a control for 20 min. In the following 20 min, infusion of either SME or ANF (all drugs dissolved in 10 mM NaCl in 5% glucose) was performed at the same rate (Mills *et al.*, 1986). The urine volume

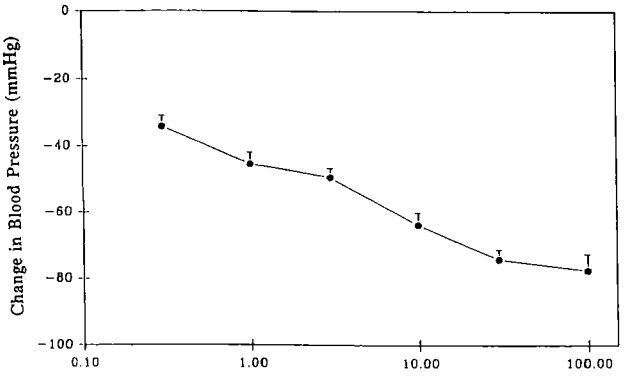

Figure 1. Log-dose response curve showing the hypotensive action of extracts of *Solanum melongena* (SME). Dose was administered in mg dry weight per kg rat body weight. Vertical bars denote SEM. $n=6$.

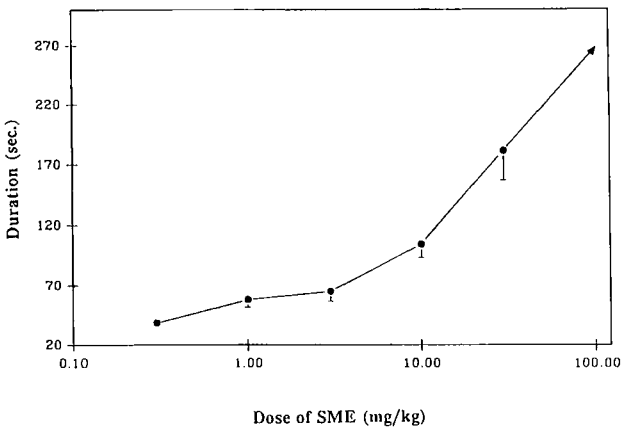

Figure 2. Duration of response recovery of the hypotensive effect of SME. The duration increased with the dose of SME but at 100 mg/kg, MAP could not recover to normal completely. $n=6$.

of the samples was measured and the sodium content was recorded by atomic absorption spectrometry.

In vitro preparation

Cardiac effect. Female rats weighing 300–350 g were killed by cervical dislocation. The entire heart was removed and rinsed in aerated (95% O_2; 5% CO_2) Krebs–Henseleit solution (K–H solution: NaCl, 115 mM; KCl, 5 mM; $CaCl_2$, 2.1 mM; $NaHCO_3$, 25 mM; NaH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; glucose 11 mM) at 37 °C. The right atrium was isolated and transferred to another petri dish with fresh K–H solution. The ends of contraction axis of the atrial tissue were tied by silk threads and suspended inside a tissue chamber containing 10 mL of aerated K–H solution at 37 °C. The threads were connected to the bottom of the tissue chamber and a force displacement transducer (Narco Myograph F-60). Isometric contractions were recorded on a Narco Physiograph (MK-III). After the atrial tissue was equilibrated for at least 30 min under a resting tension of 1 g, SME was added directly into the chamber and cumulative dose responses were obtained (Ho *et al.*, 1989).

Vasorelaxant effect. Female rats (300–350 g) were killed by decapitation. The ventral artery of the tail was isolated and quickly placed in aerated K–H solution. Pieces of artery (1.5 cm) were then cut into helical strips and suspended inside a tissue chamber containing 10 mL of K–H solution aerated with 95% O_2; 5% CO_2 at 37 °C. The two ends of the helical strip were tied with silk threads. One end was connected to the bottom of the tissue chamber and the other to a force displacement transducer (Narco Myograph F-60). Isometric contraction was recorded on a Narco Physiograph (MK-III). Before testing, the helical strip was equilibrated for about 60 min under a resting tension of 0.7 g. 60 mM KCl was used to preconstrict the helical strip. An acceptable strip should develop a tension between 0.5 and 1.0 g. Tests with KCl should be performed three times. After each test, the strip should be washed several times with K–H solution and 10 min allowed for equilibration. When a maximal level of tension was

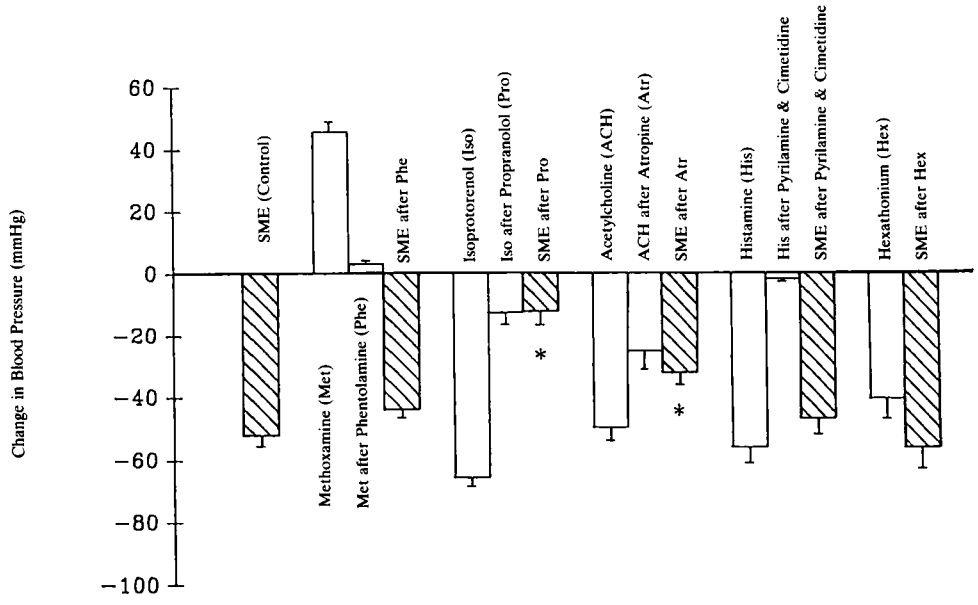

Figure 3. Effect of α- and β-adrenergic, cholinergic, histaminergic and autonomic ganglion transmission blocking agents on the hypotensive effects of SME. * represents hypotensive responses of SME significantly reduced from that of control. $n=6$ or 7.

attained under KCl, SME was added to the chamber and its relaxing effect was estimated.

All values were expressed as mean ± SEM. Student's *t*-test was used wherever applicable. The level of significance was $p<0.05$. The ED_{50} of the dose–response curves were calculated according to Fleming *et al.* (1972) and Tallarida and Jacobs (1979).

RESULTS

Solanum melongena extract produced successive hypotensive responses in normotensive rats. Those responses were dose related. The ED_{50} was 6.5 mg/kg (Fig. 1). The hypotensive responses recovered to normal, except at 100 mg/kg, and the duration of response recovery was also dose related (Fig. 2).

From the results of pharmacological antagonist studies, the hypotensive action of SME was not mediated through the autonomic ganglion transmission. After the adminstration of 3 mg/kg hexamethonium, SME (6.5 mg/kg) resulted in a 56.11 ± 6.83 mmHg decrease of blood pressure (Fig. 3). It was not significantly different from that of the control group (52.27 ± 3.51 mmHg decrease in MAP). Furthermore, the hypotensive response of SME was ascribed not to

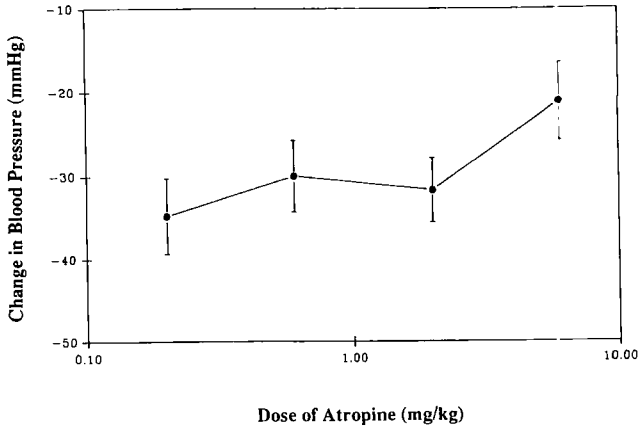

Figure 5. Effect of atropine on hypotensive action of SME. $n=5$.

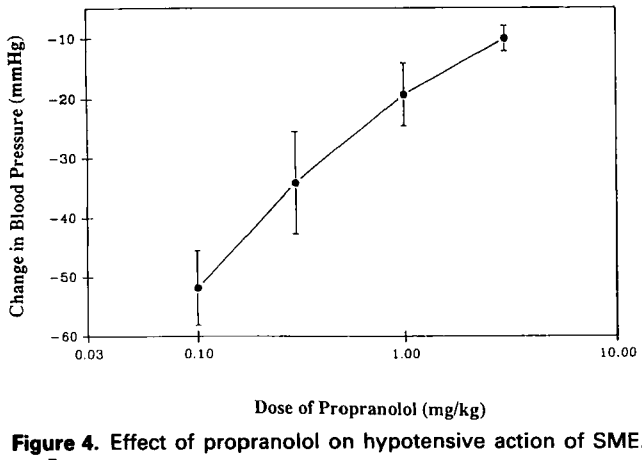

Figure 4. Effect of propranolol on hypotensive action of SME. $n=5$.

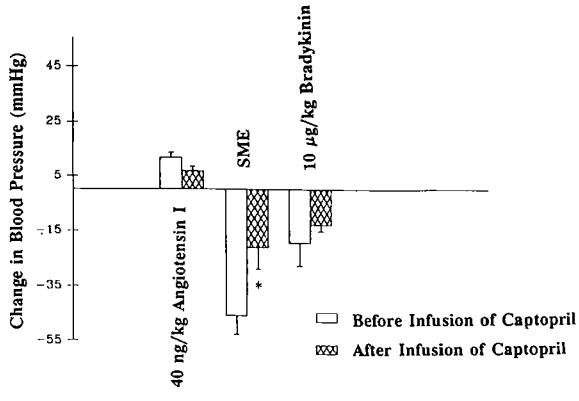

Figure 6. Effect of captopril infusion on changes in MAP with angiotensin I, SME and bradykinin. * indicates significant difference. $n=5$.

Table 1. Effect of SME infusion on urine excretion in water-loaded rats

Dose of SME	Urine vol. (mL/20 min) Control	Drug infused	% Increase
0.03 mg/min ($n=6$)	1.78 ± 0.27	1.98 ± 0.52	11.20%
0.1 mg/min ($n=6$)	2.79 ± 0.39	4.67 ± 0.85	67.00%[a]
1 mg/min ($n=6$)	3.40 ± 0.43	4.95 ± 0.83	45.60%
3 mg/min ($n=6$)	2.87 ± 0.23	5.16 ± 1.24	79.80%
500 ng/min ANF ($n=5$)	2.32 ± 0.65	3.89 ± 1.51	67.67%

[a] $p<0.05$.

be mediated through α-adrenoceptors and histaminergic receptors. In the presence of an α-adrenoceptor blocker, phentolamine and histamine blockers, pyrilamine and cimetidine, the hypotensive effect of SME remained unchanged.

Isoproterenol (1.2 μg/kg) produced a decrease in MAP of 66.13 ± 2.78 mmHg. In the presence of propranolol, it produced a drop of pressure of 12.84 ± 3.94 mmHg only, and SME produced a decrease in MAP of 12.38 ± 4.43 mmHg which is a significant reduction compared with control. In the presence of the cholinergic blocker, 2 mg/kg atropine, the change in MAP induced by 1.2 μg/kg acetylcholine reduced from 50.00 ± 4.15 to 26.78 ± 3.34 mmHg. SME diminished the change in MAP to 32.33 ± 3.91 mmHg, which also was significantly less than that of the control. This attenuation of SME-dependent MAP positively correlated with the dose of propranolol. A dose-related relationship between the change in blood pressure and dose of blocker was observed. Increase of propranolol dosage from 0.1 to 3 mg/kg correlated significantly with a change in MAP produced by SME (Fig. 4). This change showed an increase of the blocking effect with the hypotensive action of SME. Also, doses of atropine from 0.2 to 6 mg/kg correlated with a change in blood pressure produced by SME (Fig. 5).

The infusion of the angiotensin-converting enzyme inhibitor, captopril, did not induce a significant change in MAP with angiotensin I and bradykinin except SME, although the pressor and depressor effects of these drugs were attenuated. The change in the MAP response to SME was, however, significantly reduced from 45.97 ± 6.68 to 21.07 ± 7.98 mmHg (Fig. 6).

Both ANF and SME produced an increase of urine volume and sodium ion content in urine (Tables 1 and 2). At 0.1 mg/min SME, only the urine volume was significantly increased by about 67%, from 2.79 ± 0.39 mL to 4.67 ± 0.85 mL, whereas at 3 mg/min SME, a significant rise of sodium ion excretion resulted,

Table 2. Effect of SME infusion on sodium ion excretion in water-loaded rats

Dose of SME	Na ion excretion (μg/20 min) Control	Drug infused	% Increase
0.03 mg/min ($n=6$)	185.49 ± 62.22	194.89 ± 55.45	84.43%
0.1 mg/min ($n=6$)	938.51 ± 250.89	2538.86 ± 862.58	170.00%
1 mg/min ($n=6$)	783.00 ± 183.89	1229.23 ± 263.69	56.99%
3 mg/min ($n=6$)	491.81 ± 101.52	1476.14 ± 463.92	200.00%[a]
500 ng/min ANF ($n=5$)	687.10 ± 281.00	1273.13 ± 806.50	85.29%

[a] $p<0.05$.

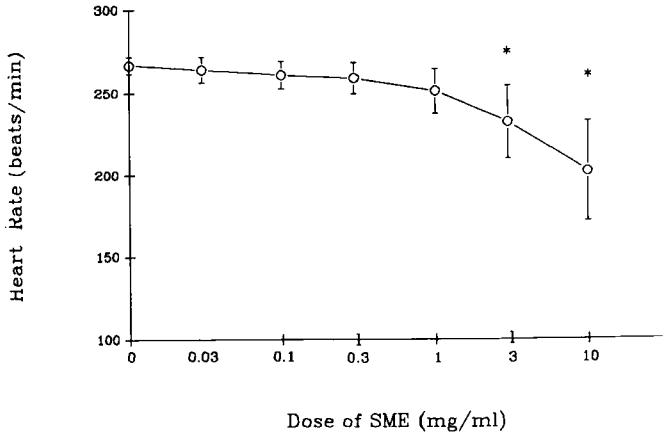

Figure 7. Chronotropic effect of SME on isolated right atria of rats. Initial atrial heart rate was 267 ± 5.08 beats/min. $n=6$. • indicates significant decrease.

compared with the control group. A 200% increase of sodium ion excretion was obtained after infusion of this dosage of SME.

In *in vitro* preparations, SME acted directly on the cardiac muscle and blood vessel muscle. Significant individual variations of the atrial tissue at high concentrations of SME were observed, as judged by the SE. SME only induced a significant drop of heart rate at high doses (Fig. 7), while the contractile force of atrial muscle decreased with the increase of dosage of SME, that is the negative inotropic effect (Fig. 8). Increased dosages of SME from 0.03 to 3 mg/mL led to a decrease in tension on tail artery strips after KCl preconstriction (Fig. 9).

DISCUSSION

The present results indicate that the aqueous crude extract of *S. melongena* fruits possesses a distinct hypotensive effect in anaesthetized normotensive rats. The dose–response study suggests that the ED_{50} is 6.5 mg/kg and this dose has therefore been used with blocker studies.

Based on data obtained in pharmacological antagonist studies, the hypotensive action of SME is not

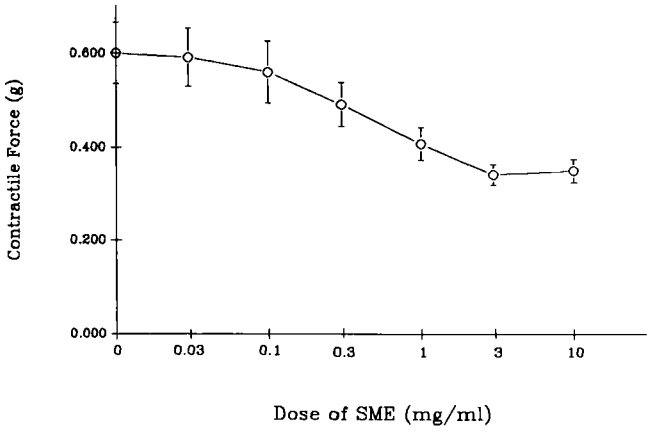

Figure 8. Intropic effect of SME on isolated right atria of rats. Initial contractile force was 0.60 ± 0.065 g. $n=6$.

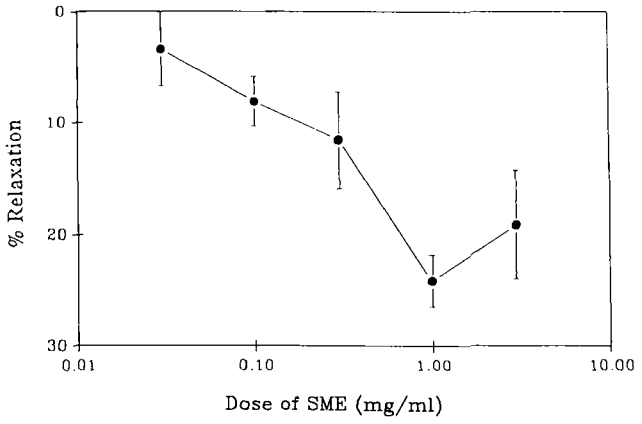

Figure 9. Vasorelaxing effect of SME on tail artery of rats. Initial tension of KCl-preconstricted helical strips was 0.54 ± 0.09 g prepared for SME testing. SME significantly relaxed tail artery preparations for all dosages tested. $n = 12$.

mediated through autonomic ganglion, α-adrenoceptor and histaminergic receptors. However, SME probably works via the β-adrenoceptor and the muscarinic cholinergic receptor stimulation to produce a hypotensive response, since the blockade of cholinergic activity suppresses the hypotensive effect of SME, whereas that of β-adrenergic activity significantly attenuates it. Attenuation of these hypotensive effects is also seen with increasing doses of these blockers inhibiting these receptors (Figs. 4 and 5). The involvement of the β-receptor in the hypotensive response receives support from the present data on the vasorelaxing effect of SME on KCl preconstricted blood vessels. Vasoconstriction due to high KCl concentration, which depolarizes the membrane, is induced by the influx of calcium ions (Antonaccio, 1984; Fleckenstein, 1977). The relaxing effect of SME might be related to an activation of the β-adrenoceptor leading to calcium ion efflux. This would activate the baroreceptors to increase cardiac output (Antonaccio, 1984).

From the present *in vitro* studies, however, SME induces a negative inotropic effect which is dose related and has only little influence on the chronotropic effect except at high doses. β-activation with isoproterenol especially invariably leads to increases in heart rate and tension (Goldberg and Rajfer, 1986). This would suggest that SME does not interact with the cardiac β-adrenoceptor. The phenomenon could possibly be attributed to SME working on the muscarinic cholinergic receptors. It has generally been proposed that the activation of the cardiac muscarinic receptor causes reduction of atrial rate and contractile force (Katzung, 1982).

Also, the hypotensive effect is probably accounted for by involvement of the renin–angiotensin system. SME might also work on the increased excretion of urine and sodium, indirectly producing a diuretic effect. Increase of excretion in rats would lead to a fall in the blood volume and a drop in the blood pressure (Pistoia and Dejey, 1977). It has been reported that a component in *S. melongena* actively aids the excretion of bile by liver, and enhanced excretion of biliary constituents therefore follows. It promotes the flow of bile into the intestine and reduces the level of cholesterol (Pistoia and Dejey, 1977).

To sum up, SME is hypotensive in normotensive rats. This response is due to the vasodilation of the resistance artery via β-adrenergic receptors, reduction in cardiac activity via muscarinic cholinergic receptors and probably a decline in the renin–angiotensin and bradykinin activity. It also acts as a diuretic agent thereby possibly reducing the blood volume (Fries, 1983), although the relationship between the mechanism of the blood pressure lowering effect and that of diuresis is not clear (Velasquez, 1986). In view of its multiple targets, SME could well be a very potent hypotensive agent.

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